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Assessing and predicting the success of *Najas flexilis* (Willd.) Rostk. & Schmidt, a rare European aquatic macrophyte, in relation to lake environmental conditions

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Key words

Najas flexilis, plant traits, fitness, macrophyte, conservation, lake.

ABSTRACT

Najas flexilis (Willd.) Rostk. & Schmidt is a submerged annual macrophyte, rare in Europe, which is protected under the EC Habitats Directive. *N. flexilis* appears to be decreasing in the British Isles, its main stronghold in Europe. We outline the environmental conditions required for *N. flexilis* growth, comparing between present and recentlyextinct sites for the plant. Plant traits (leaf area /shoot length; and reproductive number /shoot length) can be used to assess *N. flexilis* population success, and models are produced that can predict this. Both the comparison between present and extinct sites, and the models, suggest that eutrophication and acidification of lakes are the main threats to *N. flexilis*. Acidification appears to reduce the ability of *N. flexilis* to produce seeds (potentially fatal for an annual). On the other hand, eutrophication leads to conditions where *N. flexilis*, an obligate carbon dioxide utiliser, cannot photosynthesise due to the predominance of bicarbonate rather than dissolved carbon dioxide in lake water.

Introduction

Najas flexilis (Willd.) Rostk. & Schmidt is a rooted submerged macrophyte, of elodeid growth form (Den Hartog & Segal, 1964). However, unlike many other elodeids *N. flexilis* never reaches the water surface to flower and is relatively short (rarely > 0.3m in height). *N. flexilis* is an annual and has not been reported to possess any method of vegetative reproduction (Hutchinson, 1975). If there is no long-term seed bank this reliance on yearly seed production could have important repercussions for *N. flexilis* conservation in many of its threatened European habitats.

N. flexilis grows in mesotrophic lakes and is an obligate dissolvedcarbon dioxide utiliser (Hough & Fornwall, 1988; Hough & Wetzel, 1978). It has been reported from depths of 0.2 - 14.0 m (Pip and Simmons, 1986). The phosphorus requirement of the plant is supplied primarily from the sediment (Carignan and Kalff, 1987; Moeller et al., 1988).

N. flexilis has a circumpolar, boreal-montane distribution, but is found much more frequently in North America than in Europe and Asia (Hultén & Fries, 1986; Preston & Hill, 1997). It is rare enough in Europe to be listed in Annexes II and IV of the EC Habitats Directive (92/43EEC). *N. flexilis* has its European stronghold in Scotland, with 35 lake sites at which positive records have been made during 1998-2000 (“present” sites). There are eight sites in Scotland and Ireland where the plant has been recorded in the last century, but searches during 1998-2000 have failed to find it (“extinct” sites).

Due to the high conservation status of the plant and this apparent trend of extinctions, knowledge of the environmental conditions controlling plant success is required if its future conservation is to succeed. An effective method of assessing plant success within a site is also required, so that at-risk sites can be identified and appropriate action taken to prevent further extinctions.

Methods

N. flexilis presence and absence

During the course of this study, 42 lakes were investigated in Scotland (during July-September of the years 1998-2000), together with 10 lakes in Ireland (August 2000). This included all the post-1980 records for *N. flexilis* in Scotland along with all mainland Scotland pre-1980 records and a geographically-extensive range of the Irish sites. At each site *N. flexilis* presence or absence was recorded, together with a set of environmental factors likely to be relevant to the ecology of *N. flexilis* in Britain and Ireland. *N. flexilis* was searched for using snorkel, boat and shoreline searches.

Conductivity and pH were measured with Hanna portable meters, and photosynthetically active radiation (PAR) was measured with a Skye SKP210 twin-sensor meter, on site. The PAR readings were taken at the water surface and 0.5m beneath the water surface. From these readings, the light extinction coefficient was calculated using the standard formula (Kirk, 1994). One water sample per lake was collected at a sub-sample of the sites (n = 35) and analysed by the Scottish Environmental Protection Agency for the environmental variables listed in Table 1. These measurements were made after filtration, to exclude particulates. One sediment sample per lake (collected by Ekman grab) was taken in the immediate vicinity of *N. flexilis* stands (present lakes), or at a randomly-selected deep-water location (extinct lakes). All samples were collected from approximately the same depth in the sediment (approx. 0 – 5 cm). The sediment was then dried, ground and sieved to remove solid particulates greater than 2mm in size, then analysed by the Scottish Agricultural College for the environmental variables listed in Table 1.

The environmental conditions at present sites (where *N. flexilis* was found during 1998-2000) were compared to extinct sites (where *N. flexilis* was not found during 1998-2000) to determine the environmental tolerances of the species. The extinct sites were split into two groups. The first group contains the more oligotrophic extinct sites, and the second the more eutrophic extinct sites. The

environmental variables of both the extinct groups were compared to those where *N. flexilis* was present, using a Mann-Whitney U test. This test was chosen due to the small sample size in the more oligotrophic extinct group, and the non-parametric nature of the data (due to the detection limit imposed on many of the environmental variables).

N. flexilis fitness and lake environmental conditions

A method for assessing *N. flexilis* success is required to assist a monitoring programme aimed at identifying at-risk sites, to prioritise action to prevent further extinctions. A model predicting the success of *N. flexilis* could also be used to determine when sites are at risk from extinction, or when extinct sites may return to a condition suitable for reintroduction. Species abundance is often used to determine the success of a species at a particular site. However, it is difficult to measure *N. flexilis* abundance accurately. Preliminary surveys showed that *N. flexilis* could not be seen at all sites by snorkelling at the water surface. This was due to the deep water typically inhabited by *N. flexilis* and the coloration of the water (often peat-stained). Tests in clear water agreed with comments in the literature (Capers, 2000) that values of abundance obtained by grapnelling corresponded poorly with the real abundance of *N. flexilis*: the plant is consistently under-recorded by this method, as many individuals "escaped" the grapnel due to the plants' slender and fragile form. Scuba diving, or the use of underwater video cameras, is an option, but time and resource factors usually preclude the use of these methods for routine, extensive monitoring.

Instead, measures of plant attributes were used in this study to determine quantitative relationships between the environmental factors (which appeared to differ between present and extinct sites) and the success of *N. flexilis.* Plant traits have often been used to look at genotypic effect on plant fitness (eg. Dudash, 1990; Fischer & Matthies, 1998; Ouborg & Van Treuren, 1995). Plant traits have also been used to compare the fitness of species within the same genus to different environmental regimes (Sultan, 2001). The ideal fitness measure might include seed production,

probability of seed germination, probability of survival to adulthood and reproductive number of those individuals that survived to adulthood (Dudash 1990). This is not always possible. Instead, probability of the progeny reaching maturity and reproducing can be substituted by reproductive weight or vegetative success of the reproducing parent, as this represents the resources allocated, or available, to the offspring (Sultan, 2001). Such fitness traits are phenotypically plastic within genotypic constraints. This plasticity will be determined by the way in which the genotype of the organism interacts with its environment. It is this environmentally-driven plasticity which can be used to determine the realised niche of *N. flexilis* in Britain and Ireland, as the patterns of fitness response (along with the growth traits that underlie them) determine the range of conditions in which species may survive and successfully reproduce, and hence their ecological niche-breadth (Sultan, 2001). Phenotypic plasticity has been successfully related to environmental variables in the field including specific leaf area and depth as a function of light (Spence *et al*., 1973).

Three whole-plant samples were randomly collected from 17 Scottish populations, and trait analysis was undertaken on the fresh plants. Leaf area was measured for each plant using the method of Ali *et al*. (1999). Leaf area measurements are known to be a good surrogate for the photosynthetic success of plants, and hence the resources available to be allocated to the progeny. Number of reproductive structures was also recorded, to represent the reproductive success of the individuals. A problem with relating wild population plant traits to environmental factors is that they can be affected by the age of the individual, which is often unknown in wild perennial populations. However, this problem does not exist when measuring plant traits of an annual, such as *N. flexilis* the established-phase of which lives only for a maximum of one year. To account for the differences found in plants collected at the beginning, to those at the end of the field season all traits were divided by shoot length, a function of age.

Data were tested for normality using Ryan-Joiner test procedures, and appropriate transformations

were applied to normalise individual data sets. Multiple regression procedures were used to relate *N. flexilis* fitness to the suite of environmental variables listed in Table 1.

Results

N. flexilis presence and absence

The Mann-Whitney U tests showed a significant difference between both extinct groups and the present group (Table 1).

N. flexilis fitness and lake environmental condition

In unfavourable conditions for *N. flexilis* growth, the two elements of fitness used, reproductive number and leaf area, did not reduce uniformly together. In more oligotrophic lakes (pH <7.0) leaf area was intermediate, but the number of reproductive structures reduced dramatically. At one site only one seed was found in three mature plants. This agrees with the results of the experiments by Titus & Hoover (1993). In other environmentally similar lochs, only one or two plants were found, so they could not be sampled for fitness. It is probable that this low frequency of plants and low seed frequency among plants is related. At the more eutrophic end of the range (pH > 8.0), plants appeared to have longer internodes, which reduced the total leaf area of the plants as there were less leaf nodes and therefore less leaves per shoot length (overall shoot length did not appear to increase in plants with extended internodes). The lack of nodes also reduced the number of reproductive structures as these develop in the leaf nodes. Both of these conditions could possibly lead to population collapse. This is illustrated in Fig. 1a & 1b, where each fitness component is regressed against pH. Both of the plots are noisy (as pH is not the only factor controlling fitness). However they illustrate the unequal decline in the two components of plant fitness. As a result of this situation (reproductive number/shoot length) x (leaf area/shoot length) is used as the measure of fitness and is the measure predicted by the resulting models.

Predicting *N. flexilis* fitness

The models for leaf area/shoot length and number of reproductive structures/shoot length are intrinsically similar. This is to be expected as the leaves provide the resources for the reproductive structures. Light extinction coefficient is a good indicator for predicting photosynthetic success as leaf area/shoot length. However, it is not as good for predicting the number of reproductive structures produced. The quantity of calcium in the sediment is a better indicator. This is probably because it will affect the pH of the water, which appears to affect reproductive success.

Model 1: $r^2 = 0.886$ $p < 0.005$

logLa/sl = 0.515 + (1.276logAlk) - (1.012logPs) + (50.775TPw) - (0.0921Mgw) - (0.599logLec)

Model 2: $r^2 = 0.672$ $p < 0.005$

Rep/sl = 0.993 + (2.154logAlk) - (1.179logPs) + (44.801TPw) - (0.132Mgw) - (0.856logCas)

Model 3: $r^2 = 0.768$ $p < 0.005$

logFit = -0.628 + (2.263logAlk) - (1.810logPs) + (84.067TPw) - (0.156Mgw) - (0.919logLec)

Model 4: $r^2 = 0.743$ $p < 0.005$

logFit = 0.642 + (2.738logAlk) - (1.392logPs) + (59.509TPw) - (0.199Mgw) - (0.649logCas)

Where:
La/sl = Leaf area/shoot length
Fit = (Leaf area/shoot length) x (Reproductive structure number/shoot length)
Rep/sl = Number of reproductive structures/shoot length
Alk = Alkalinity mg/l
Ps = Extractable phosphorus in the sediment mg/l
TPw = Total phosphate in the water mg/l
Mgw = Magnesium in the water mg/l
Cas = Calcium in the sediment mg/l
Lec = Light extinction coefficient

The models created were tested using four Irish sites and four Scottish sites not previously used in the creation of the models. The predicted values were plotted against the observed values (Fig. 2). The model predicting reproductive success alone works poorly. All the other models appear to predict the fitness of *N. flexilis* quite well. The major exception from the test data is from Lough Leane in Ireland where the fitness of the plant is consistently predicted to be greater than the observed fitness. Lough Leane is presently becoming increasingly eutrophic. This recent change

may well be affecting the plants, but it is possible that phosphate will not yet have accumulated in the sediment, hence the over-prediction of plant fitness. These results show that, with the exception of Lough Leane, the models can equally be applied to the Irish and Scottish *N. flexilis* populations. Therefore they could be used to predict which *N. flexilis* sites are at risk and which have recovered to a point where reintroduction is possible.

Discussion

There is an overlap between the ranges of the environmental variables of the present and extinct site groups. This is because the population at the extinct site could have become extinct at any time since the last record, in some cases a period of over 100 years. Since the time of extinction, the lake quality may have improved to a state where *N. flexilis* could theoretically survive, but recolonisation may not have occurred. Another possible reason why the ranges for the present and extinct site groups are not discrete is that the environmental variables do not affect *N. flexilis* independently and extinctions will be brought about by a number of contributing factors.

The water chemistry measurements are a snapshot of the lake water chemistry. The nutrient levels in the samples are low, as they were taken in summer, when most of the nutrients will be incorporated in the growing plant and/or cyanobacterial material. However, it is within this time period that *N. flexilis* grows, so this is an appropriate time period to take the measurements. Many of the dissolved reactive phosphate, total oxidised nitrogen and NH_3 concentrations were below the detection limit, which undoubtedly contributed to the fact that no significant difference was found between these nutrient levels in the present and extinct groups, and their effect on the fitness of *N. flexilis* could not be determined.

The models, and the present extinct site comparisons, suggest that eutrophication and acidification are the main threats to *N. flexilis*. They both suggest that phosphate concentration in the water and

sediment are important, as are calcium and alkalinity. The conditions that predominate in the more oligotrophic sites appear to affect the reproductive success of the plants, but not their photosynthetic capacity. Unless the plant is preferentially allocating resources to photosynthetic tissue instead of the reproductive structures (which is an unlikely strategy for an annual plant), this suggests that these environmental conditions are inhibiting reproductive development alone. Experiments on floral development and pollen viability in these conditions are required to confirm this.

Alkalinity, pH and calcium levels in the water are all indicators of the concentration of dissolved inorganic carbon in the water. Higher levels of these measures indicate that the predominant form of inorganic carbon in the water is bicarbonate. These measures were all found to be significantly higher in the eutrophic extinct group than the present group. The pH, alkalinity and calcium levels and also the inorganic carbon equilibrium will be determined, in part by, the characteristics of the catchment, but they will also shift in accordance with biological activity within the lake. High levels of photosynthesis by either algae or macrophytes within a lake will tend to shift the equilibrium towards a bicarbonate dominated system, at least in daytime when photosynthesis is occurring. *N. flexilis* is an obligate CO_2 user and cannot utilise bicarbonate (Hough & Fornwall, 1988).

High levels of photosynthesis in aquatic plants require high nutrient levels to sustain them. As nitrogen and phosphorus often limit primary production in mesotrophic lakes (where *N. flexilis* grows) an increase in these nutrients would increase photosynthetic production, which would in turn decrease available dissolved CO_2 concentrations. This would seriously limit the growth of *N. flexilis*, since in such an environment it would be incapable of fixing carbon. The significantly higher levels of phosphate in the water and the sediment and the higher pH, alkalinity and calcium levels, in the more eutrophic extinct sites, suggest that this may be the cause of *N. flexilis* extinction in these lakes. The extinct eutrophic sites in this study are all found in the more intensive agricultural areas, suggesting that phosphate inputs from agriculture in the catchments of these lakes

may be a serious threat to the species.

Models 1 and 3 show that underwater light availability is also important for predicting plant success. The quantity of photosynthetically active radiation reaching *N. flexilis* plants will decrease as algal and macrophyte growth increases, because these will intercept the light before it reaches the relatively low-growing *N. flexilis*. However, *N. flexilis* naturally grows at low light levels and there is no significant difference in the light extinction coefficient between present and absent sites. This suggests that light would have to be severely limited to cause *N. flexilis* extinction.

Conclusions

The models and analysis of present and extinct sites, have suggested that the major threats to the species in Scotland are those related to eutrophication and acidification. Both of which can cause population extinction. The fitness measures developed here can be used to monitor and assess the present *N. flexilis* populations and elucidate which populations are at risk. The models can be used to calculate the predicted fitness of *N. flexilis* at extinct sites to determine if the lake quality is suitable for reintroduction of *N. flexilis*.

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Tables

Table 1 The range of environmental variables measured at *Najas flexilis* present and extinct sites, a) in the sediment, b) in the water. A * denotes a significant difference between the extinct group with

the * and the present group, $p \leq 0.05$, using a Mann-Whitney U test.

a)

	Oligotrophic extinct	Present	Eutrophic extinct
Available P (mg/l)	0.9 - 2.9 (n = 2)	0.3 - 17.3 (n = 41)	5.3 - 72.9* (n = 7)
Median	1.9	2.6	21.8
Available K (mg/l)	--	4 - 166 (n = 31)	28.3 - 89.2 (n = 4)
Median	--	35.1	77.1
Extractable Mg (mg/l)	--	16.3 - 388 (n = 31)	54.1 - 189 (n = 4)
Median	--	87	146
Extractable Ca (mg/l)	145 - 5250 (n = 2)	198 - 331000 (n = 41)	805 - 25000 (n = 7)
Median	2697.5	950	3140
Total Fe (mg/kg)	27600 - 20600* (n = 2)	94.4 - 168000 (n = 41)	123 - 14500 (n = 7)
Median	24100	1250	216
Total N (mg/kg)	320 - 6220 (n = 2)	240 - 142000 (n = 41)	600 - 11400 (n = 7)
Median	3270	3320	1540
Extractable Na (mg/l)	--	8.3 - 300 (n = 29)	20.5 - 72.5 (n = 4)
Median	--	52.4	50.05

b)

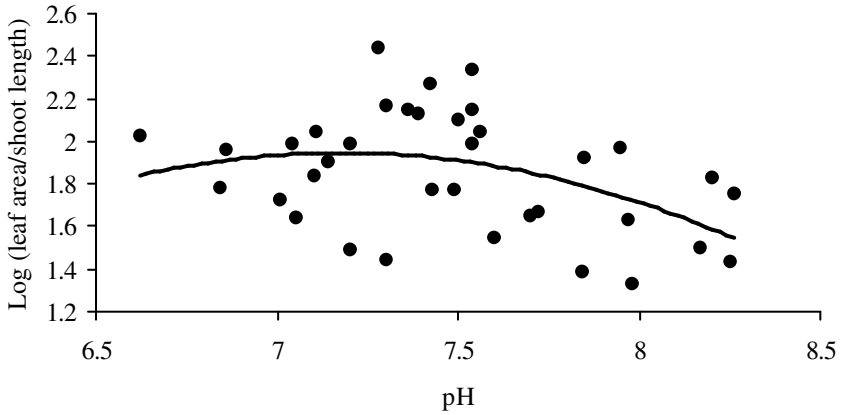
	Oligotrophic extinct	Present	Eutrophic extinct
pH	6.46 - 6.98* (n = 2)	6.62 - 8.3 (n = 42)	7.45 - 9.01* (n = 7)
Median	6.72	7.46	8.64
Conductivity (us/cm)	54.9 - 116* (n = 2)	55 - 447 (n = 42)	158 - 410 (n = 7)
Median	85.45	235	215
Light extinction coefficient	1.35 - 4.4 (n = 2)	0.53 - 7.32 (n = 42)	0.91 - 5.18 (n = 7)
Median	2.88	1.91	1.83
Alkalinity (mg/l)	4.91 - 5.7* (n = 2)	6.71 - 69.71 (n = 29)	41.5 - 98.66* (n = 3)
Median	5.3	23.45	72.65
Ca (mg/l)	1.32 - 2.02* (n = 2)	2.06 - 33.4 (n = 30)	14.22 - 24.72* (n = 3)
Median	1.67	9.59	21.7
Fe (mg/l)	0.2 - 0.16 (n = 2)	<0.03 - 1.69 (n = 30)	0.04 - 0.35 (n = 3)
Median	0.18	0.21	0.18
Mg (mg/l)	1.54 - 2.12 (n = 2)	1.34 - 8.8 (n = 30)	6.4 - 12* (n = 3)
Median	1.83	4.8	8.8
N-NH3 (mg/l)	< 0.04 (n = 2)	<0.04 - 0.1 (n = 33)	<0.04 - 0.28 (n = 4)
Median	<0.04	<0.04	0.07
Total oxidised N (mg/l)	<0.1 (n = 2)	<0.1 - 1.369 (n = 33)	<0.1 - 2.646 (n = 4)
Median	<0.1	<0.1	<0.1
Dissolved reactive P (mg/l)	<0.003 (n = 2)	<0.003 - 0.045 (n = 33)	<0.003 - 0.074 (n = 4)
Median	<0.003	<0.003	0.012
Total P (mg/l)	0.012 (n = 2)	<0.003 - 0.056 (n = 30)	0.014 - 0.16* (n = 3)
Median	0.012	0.0095	0.017
K (mg/l)	0.3 - 0.54* (n = 2)	0.16 - 2.82 (n = 33)	0.3 - 2 (n = 4)
Median	0.42	1.2	1.4

Figure legends

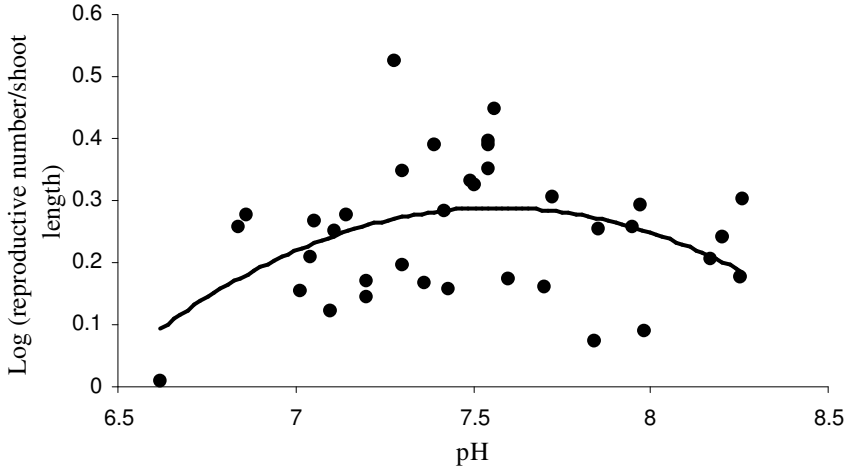
Fig. 1 a) The effect of pH on Log (leaf area/shoot length) $r^2 = 0.181$, $p < 0.05$. b) The effect of pH on Log (reproductive number/shoot length) $r^2 = 0.165$, $p = 0.052$

Fig. 2 The observed and predicted fitness values of *Najas flexilis* for 4 Irish sites and 4 additional Scottish sites, 3 in S. Uist and 1 in Kintyre, a) = model 1, b) = model 2, c) = model 3, d) = model 4.

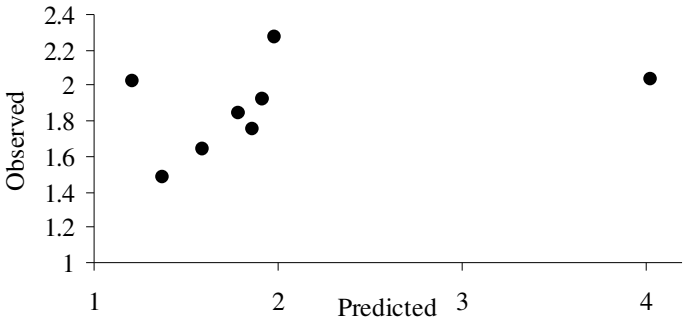
a)


Log (leaf area/shoot length)
2.6
2.4
2.2
2
1.8
1.6
1.4
1.2
6.5
7
7.5
8
8.5
pH

b)


Log (reproductive number/shoot length)
0.6
0.5
0.4
0.3
0.2
0.1
0
6.5
7
7.5
8
8.5
pH

a)


Observed
Predicted
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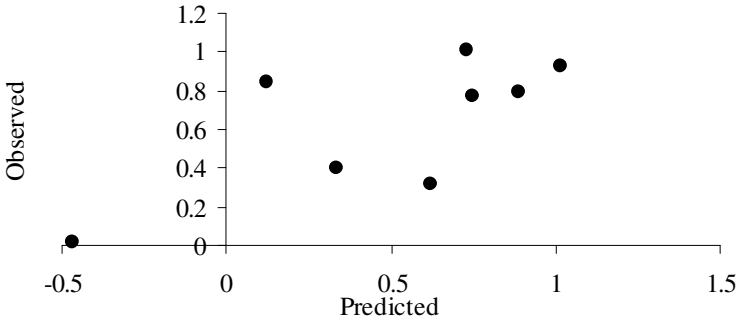
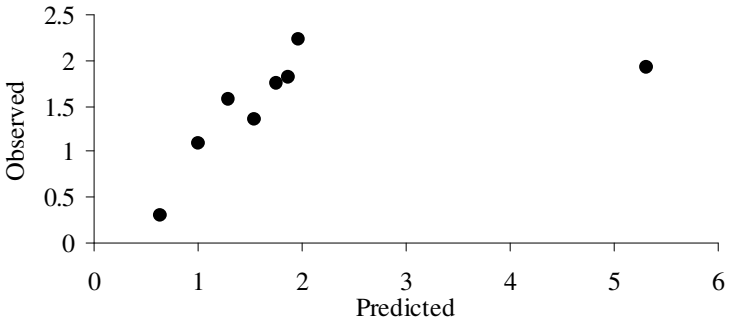
2.2
2
1.8
1.6
1.4
1.2
1
1
2
3
4

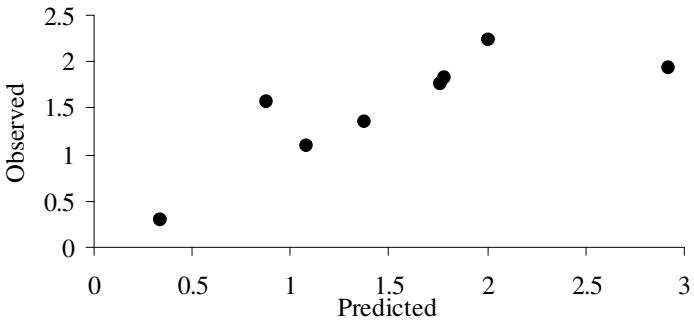
b)


Observed
Predicted
1.2
1
0.8
0.6
0.4
0.2
0
-0.5
0
0.5
1
1.5

c)


Observed
Predicted
2.5
2
1.5
1
0.5
0
0
1
2
3
4
5
6

d)


Observed
Predicted
2.5
2
1.5
1
0.5
0
0
0.5
1
1.5
2
2.5
3